

## Two Newly Identified *Gymnosporangium* Species, *G. japonicum* and *G. cornutum*, in Korea

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(Received on August 21, 2003; Accepted on October 14, 2003)

Two *Gymnosporangium* species, *G. japonicum* and *G. cornutum*, causing cedar-apple rust, were newly identified in Korea, and a new aecial host for each fungus was found by artificial inoculation. Detailed descriptions of the species were made based on macroscopic features (symptoms and signs) and light and scanning electron microscopic observations of spores and peridial cells. *G. japonicum* from *Juniperus chinensis* var. *horizontalis* had *Photinia villosa* as its new aecial host. *G. cornutum* from *J. rigida* showed its aecial stage on *Sorbus alnifolia*.

**Keywords :** Cedar-apple rust, *Gymnosporangium cornutum*, *Gymnosporangium japonicum*, *Photinia villosa*, *Sorbus alnifolia*.

In the 20<sup>th</sup> century, taxonomic studies on the genus *Gymnosporangium* have been mainly focused on host ranges and light microscopic characters of spores and peridial cells (Hiratsuka, 1936a-e; Hiratsuka and Hiratsuka, 1980; Hiratsuka et al., 1992; Kern, 1973; Parmelee, 1965, 1971; Peterson, 1982; Wang and Lin, 1985). Hence, a need for detailed description of the fungal structures using electron microscopy was recognized to update fungal taxonomy especially in the Uredinales (Hiratsuka, 1971; Hiratsuka and Kaneko, 1975; Katsuya et al., 1980; Sato and Sato, 1982; Lohsomboon et al., 1990). It was suggested that fine surface structures of aeciospores and peridial cells in the genus *Gymnosporangium* could be important diagnostic parameters in identifying its species (Lee and Kakishima, 1999a, b; Lee et al., 1999).

*Gymnosporangium* species are major pathogenic fungi causing cedar-apple rust in *Juniperus* and in pomaceous plants of the Rosaceae (Agrios, 1997). *Gymnosporangium* is mainly distributed in the northern hemisphere, having about 60 species with 15 *Roestelia* species as anamorphs (Kern, 1973). In China, 18 *Gymnosporangium* spp. and 8 *Roestelia* spp. have been recorded (Zhuang, 1994), while 9

*Gymnosporangium* spp. have been identified in Japan (Hiratsuka et al., 1992). In Korea, five *Gymnosporangium* spp. including *G. asiaticum*, *G. clavariiforme*, *G. miyabei*, *G. yamadae*, and *G. shirainum* have been recorded (Hiratsuka, 1935, 1940; Takimoto, 1916; Park, 1958). However, collections for *G. miyabei* and *G. shirainum* have not been made since their first report.

During the survey of cedar apple-rust, we found two species of *Gymnosporangium*, *G. cornutum* and *G. japonicum*, unrecorded in Korea. This report presents the detailed descriptions of the species observed by light microscopy (LM) and scanning electron microscopy (SEM). Aecial host ranges of the rust fungi were also investigated based on specimen collections and by artificial inoculation.

### Materials and Methods

**Collection of specimens.** A total of 28 telial and 18 aecial specimens of *Gymnosporangium* species were collected from various regions of Korea from 1984 to 2002, and deposited at the Herbarium of the Korea Forest Research Institute (HKFRI). Eight dry specimens were provided by the National Mycological Herbarium of Biosystematics Research Institute (DAOM) and the Mycological Herbarium, the Institute of Microbiology, Academia Sinica, China (HMAS). Fresh telial materials were collected from various regions in Korea during a recent survey from 1999 to 2001, and used as inocula on aecial hosts, from which fresh aecial materials were also obtained for morphological examinations by LM and SEM.

**Inoculation of teliospores on aecial hosts.** Fresh telia were collected from *Juniperus* species during the spring season of 2001 in Seoul and its vicinities and stored in a refrigerator at about 4°C until use. Teliospore inoculum was made and inoculated on Rosaceous plants by the procedures of Pearson et al. (1977) and Aldwinkle et al. (1977).

Teliospores from telia in branches and trunks of *Juniperus* trees were suspended in sterile water, and sufficiently sprayed onto the leaves of Rosaceous plants such as *Amelanchier asiatica* (Siebold et Zucc.) Endlicher, *Chaenomeles sinensis* Koehne, *C. lagenaria* (Loiselur) Koidzumi, *Crataegus pinnatifida* Bunge, *Eriobotrya japonica* (Thunb. ex Murray) Lindley, *Pyrus pyrifolia* var. *culta* Nakai, *P. ussuriensis* Maxim., *Sorbus commixta* Hedl., *S. alnifolia* (Siebold et Zucc.) K. Koch, *Photinia glabra* (Thunb. ex Murray)

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Maxim., *P. villosa* (Thunb. ex Murray) DC., *Malus sieboldii* (Regel) Rehder, and *M. pumila* Mill. These species have been recorded as aecial host plants of *Gymnosporangium* (Kern, 1973; Lee, 1999). Two- to three-year-old Rosaceous plants, three per each species, were used in this study. The plants were placed in two humidified chambers at room temperature for 2-3 days.

**Light microscopy (LM).** Light microscopic characters of specimens examined under a compound light microscope (Axiophot, Zeiss, Germany) included shape and size of peridium and peridial cells; shape, size, and color of aeciospores; and thickness of cell walls of aeciospores and teliospores. Aecial specimens obtained from artificial inoculation on Rosaceous plants were also compared with those of natural collections.

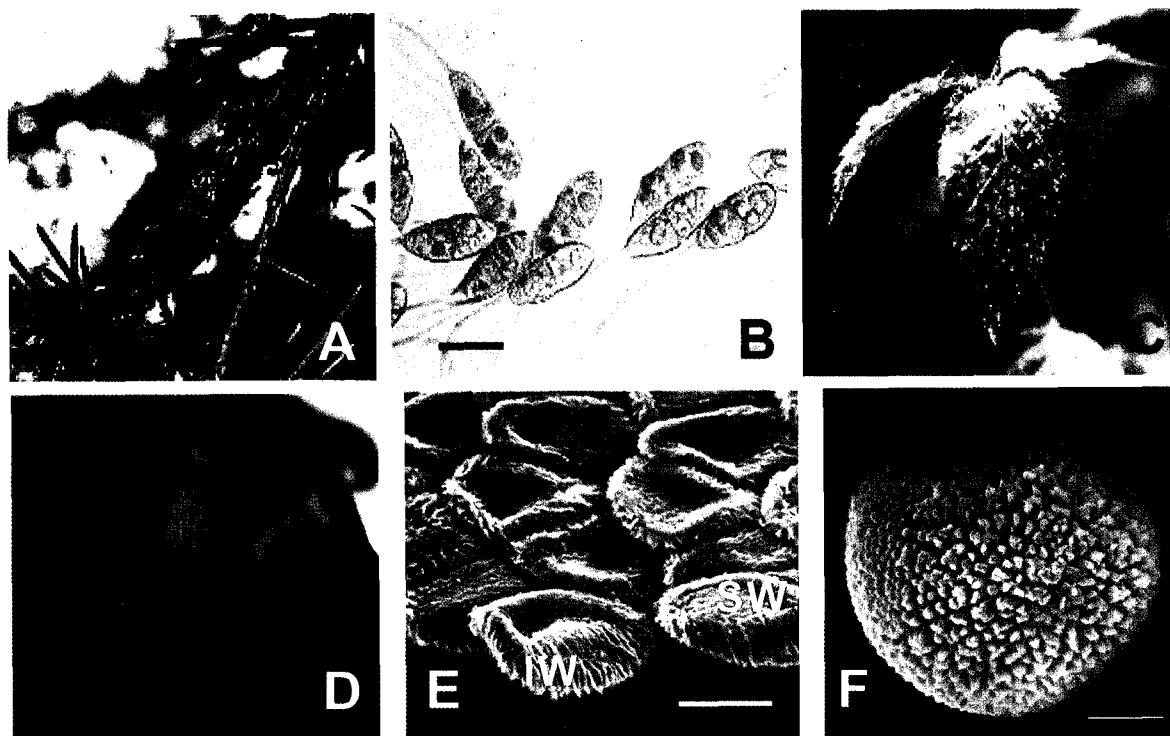
**Scanning electron microscopy (SEM).** Fine surface structures of aeciospores and peridial cells were examined by SEM. Aeciospores and peridial cells obtained from natural host and infected plants by artificial inoculation were dusted on double-sided adhesive tapes on specimen holders and then coated with gold using a Hitachi E-1010 Ion Sputter, and then examined under an S-3000N SEM (Hitachi, Japan) at 15 kV. Types of the aeciospore surface structures and peridial cell structures were identified according to Lee and Kakishima (1999a, b).

**Identification and description of species.** Identification and description of the cedar-apple rust fungi were based on the data from previous studies (Kern, 1973; Hiratsuka et al., 1992; Lee and Kakishima, 1999a, b), while scientific names of host plants were based on Lee (1999), Chang (1994), and Krussmann (1985).

## Results and Discussion

***Gymnosporangium cornutum* Arth. ex Kern.** Telia caulicolous formed irregular fusiform swellings of the smaller branches (Fig. 1A), applanate and low, or pulvinate, and dark-brown. Teliospores were two-celled, rarely three-celled, globoid,  $16\text{-}29 \times 36\text{-}63 \mu\text{m}$  (Fig. 1B), regular, thin-walled,  $1.4\text{-}2.8 \mu\text{m}$ , with one to two pores near the septum or one apical in the upper cell, and covered ellipsoid with hyaline papillae. Spermogonia and aecia foliicolous (Fig. 1C, D) were also possibly fructicolous, while aecia roestelioid caused some hypertrophy. Peridia were cornuted, rupturing at apex, becoming rather lacerate but retaining tubular form,  $0.5\text{-}2.5 \text{ mm}$  high, while peridial cells were rounded rhomboidal,  $48\text{-}101 \mu\text{m}$  long, outer cell smooth (type S), side cells moderately rugose (type MR), and inner cells small papillate (Type SP) (Fig. 1E). Aeciospores were globoid,  $17\text{-}27 \times 18\text{-}28 \mu\text{m}$ , walls yellow-brown,  $1.3\text{-}2.5 \mu\text{m}$  thick, and minutely coronate (Type MC) (Fig. 1F).

**Specimens examined:** O, I on *Sorbus alnifolia* (Siebold et Zucc.) K. Koch, Gwanak-gu, Seoul, 8 July 2000, H. Y. Yun (HKFRI 2071); Boeun-gun, Chungbuk, 15 July 1999, S. K. Lee (HKFRI 931); Nowon-gu, Seoul, 20 June 1998, S. K. Lee (HKFRI 928, 935); 20 June 2000, H. Y. Yun [HKFRI 2053, a result of teliospore inoculation of *G. cornutum*



**Fig. 1.** Macroscopic and microscopic features of *Gymnosporangium cornutum*. A: Telia on *J. rigida*. B: Teliospores ( $\times 400$ ) (scale bar:  $20 \mu\text{m}$ ) C: Artificially-induced spermogonia on *S. alnifolia*. D: Artificially-induced aecia on *S. alnifolia*. E: Surface structures of peridial cells (IW: inner wall, SW: side wall). F: Surface structure of an aeciospore. (scale bars: E =  $15 \mu\text{m}$ , F =  $5 \mu\text{m}$ )

**Table 1.** Result of artificial inoculation of teliospores of two *Gymnosporangium* species on 13 Pomaceous plants<sup>a</sup>

Aecial host plant	Rust fungal species	
	<i>G. japonicum</i>	<i>G. cornutum</i>
<i>Amelanchier asiatica</i>	– <sup>b</sup>	–
<i>Chaenomeles sinensis</i>	–	–
<i>Chaenomeles lagenaria</i>	–	–
<i>Crataegus pinnatifida</i>	–	–
<i>Eribotrya japonica</i>	–	–
<i>Malus sileboldii</i>	–	–
<i>Malus pumila</i>	–	–
<i>Photinia glabra</i>	–	–
<i>Photinia villosa</i>	+	–
<i>Pyrus pyrifolia</i> var. <i>culta</i>	–	–
<i>Pyrus ussuriensis</i>	–	–
<i>Sorbus alnifolia</i>	–	+
<i>Sorbus commixta</i>	–	–

<sup>a</sup>These woody Rosaceae species are known as aecial hosts of *Gymnosporangium* spp. in Korea.

<sup>b</sup>+ means “successively forming spermogonia and ascia” and – “not forming spermogonia and ascia”.

(HKFRI 2018)]; O, I on *Sorbus aucuparia* L., 4 September 1977 (DAOM 166487); O, I on *Sorbus decora*, September 1951 (DAOM 920471); III on *Juniperus rigida* Sieb. et Zucc., Gwacheon, Gyeonggi, 10 April 2001, H. Y. Yun (HKFRI 2018); 6 April 2000, S. K. Lee (HKFRI 1794); 15 April 2000, S. K. Lee (HKFRI 1792); 22 April 2000, S. K. Lee, (HKFRI 1793); 22 April 2000, S. K. Lee (HKFRI 1788); 3 May 2001, H. Y. Yun (HKFRI 1985); 28 March 2002, H. Y. Yun (HKFRI 1984); Gwanak-gu, Seoul, 5 May 2000, S. K. Lee (HKFRI 517), K. H. Kim (HKFRI 518, 519, 520); 20 June 1961 (HMAS 44512).

**Hosts in Korea:** O, I on *Sorbus alnifolia* (Sieb. et Zucc.) K. Koch. III on *Juniperus rigida* Sieb. et Zucc.

**Geographical distribution:** *G. cornutum* was not common in Korea and could be collected only from two locations, Seoul and Boeun.

**Remarks:** As shown in Table 1, the artificial inoculation of teliospores collected from *J. rigida* resulted in the successful production of spermogonia and ascia only on leaves of *S. alnifolia* among 13 tested aecial host trees. Although other *Sorbus* spp. are also aecial hosts of *G. cornutum*, they are not grown in Korea. Therefore, host alternation of *Gymnosporangium* species between *J. rigida*,

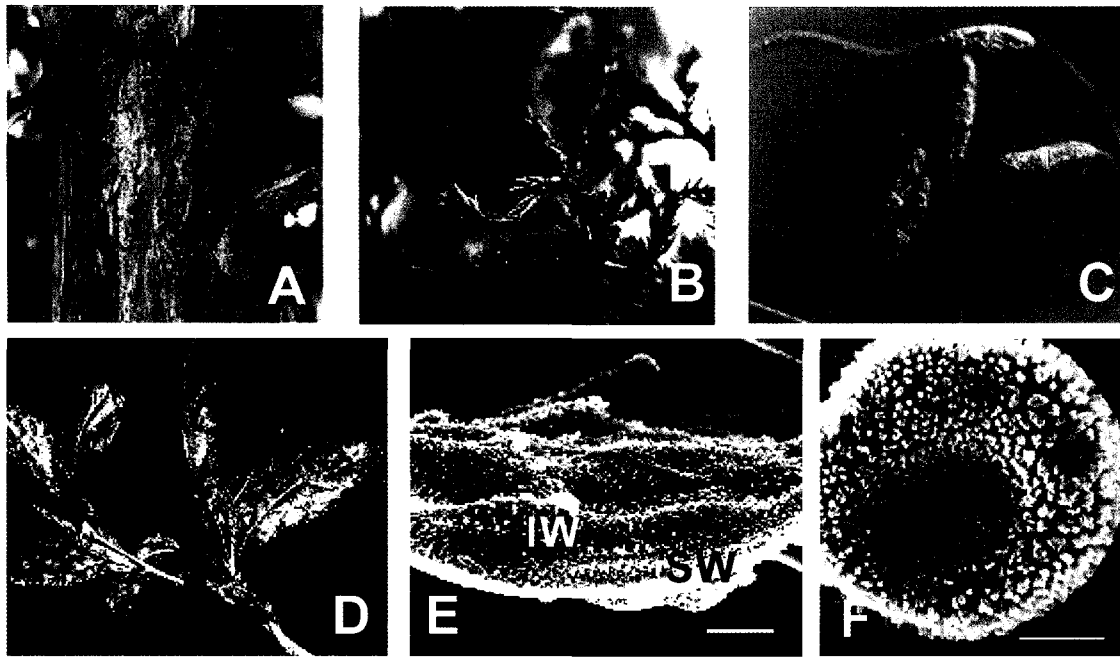
**Table 2.** Morphological characteristics of teliospores, aeciospores, and peridial cells of *Gymnosporangium cornutum*

Structure	Present study	Hiratsuka et al. (1992)	Kern (1973)	Lee and Kakishima (1999)
Telium	Caulicolous, on smaller branches	Chiefly caulicolous, foliicolous	Chiefly caulicolous, foliicolous on smaller branches,	– <sup>c</sup>
Teliospore				
Cell number	2, rarely 3	2	2	–
Shape	Globoid	Ellipsoid	Ellipsoid	–
Size (µm)	16-29 × 36-63	12.5-30 × 25-55	15-24 × 30-58	–
Wall thickness (µm)	1.4-2.8	1.0-2.5	1.0-2.0	–
No. of pores	1-2 near septum or 1 apical in upper cell	1-2 near septum or 1 apical in upper cell	1-2 near septum or 1 apical in upper cell	–
Aeciospore				
Type of surface Structure	MC <sup>a</sup>	–	–	MC
Wall thickness (µm)	1.3-2.5	2.0-2.5	1.8-3.0	2.0-2.5
Size (µm)	17-27 × 18-28	18-27 × 21-31	16-25 × 21-29	16-25 × 21-29
Peridial cells				
Type of surface Structure <sup>b</sup>				
OW	S	–	–	S
SW	MR	–	–	MR
IW	SP	–	–	SP
Length (µm)	48-101	40-110	60-110	–

<sup>a</sup>MC: type MC (minutely coronate; Lee and Kakishima, 1999a).

<sup>b</sup>OW: outer wall; SW: side wall; IW: inner wall; MR: type MR (Moderately rugose); S: type S (smooth); SP: type SP (small papillae; Lee and Kakishima, 1999b).

<sup>c</sup>Not described.



**Fig. 2.** Macroscopic and microscopic features of *Gymnosporangium japonicum*. **A:** Telia on *J. chinensis* (on trunk). **B:** Telia on *J. chinensis* var. *horizontalis* (on branch). **C:** Teliospores ( $\times 400$ ) (Scale bar: 30  $\mu\text{m}$ ) **D:** Artificially-induced spermogonia on *P. villosa*. **E:** Surface structures of peridial cells (IW: inner wall, SW: side wall). **F:** Surface structure of an aeciospore. (scale bars: E = 10  $\mu\text{m}$ , F = 5  $\mu\text{m}$ ).

a telial host, and *S. alnifolia*, a pomaceous aecial host, was verified for the first time in Korea. No significant differences were noted between the present and the previous descriptions (Table 2). However, size of peridial cells of this rust fungus was relatively smaller as compared to those of *G. cornutum* reported in other countries (Kern, 1973; Hiratsuka et al., 1992). Fine surface structure of the aeciospores was minutely coronate type (Type MC). Fine surface structures of peridial cells were smooth (type S) on outer walls, moderately rugose (type MR) on side walls, and small papillate (SP) on inner walls (Figure 1, Table 2).

***Gymnosporangium japonicum* P. Sydow.** Telia caulicolous on twigs, branches and trunks, were irregularly wedge-shaped, 3–6 mm high, and orange-brown (Fig. 2A, B). Teliospores were two-celled or rarely three-celled, ellipsoid and long-ellipsoid, 13–22 $\times$ 36–70  $\mu\text{m}$ , walls pale orange-brown, 1.0–2.7  $\mu\text{m}$  thick, usually with one or two pores near septum (Fig. 2C). Spermogonia and aecia were foliicolous and caulicolous (Fig. 2D); aecia hypophyllous, roestelioid, 2–5 mm high; peridia dehiscent at apex, retaining somewhat tubular shape; peridial cells linear rhomboid, 62–117  $\mu\text{m}$  long, outer walls smooth (type S), inner and side walls sparsely echinulate (type SE) (Fig. 2E); and aeciospores globoid, 16–21 $\times$ 20–26  $\mu\text{m}$ , walls dark yellow, 1.1–2.8  $\mu\text{m}$  thick, small coronate (type SC) (Fig. 2F).

**Specimens examined:** O. I on *Photinia villosa* (Thunb. ex

Murray) DC., Jeju-si, Jeju-do, 13 July 1999, S. K. Lee (HKFRI 694); Jeju-si, Jeju-do, 29 July 1999, S. K. Lee (HKFRI 695); Jeju-si, Jeju-do, 29 August 2001, H. Y. Yun (HKFRI 142, 144, 145, 147); Jeju-si, Jeju-do, 29 August 2001, H. Y. Yun (HKFRI 148), 23 August 2001 (HKFRI 2054), 20 June 2001, H. Y. Yun (HKFRI 2055, a result of teliospore inoculation of *G. japonicum* (HKFRI 2019)); 20 June 2001, H. Y. Yun [HKFRI 2056, result of teliospore inoculation of *G. japonicum* (HKFRI 2020)]. O. I on *Photinia laevis* var. *villosa*, 22 June 1934 (SAC 14); 21 June 1934 (SAC 5). O. I on *Photinia parvifolia*, 26 June 1932 (HMAS 11147). III on *Juniperus chinensis* L., Kangneung, Kangwon, 9 April 1988, S. K. Lee (HKFRI 504); Jeju-si, Jeju-do, 9 April 2002, S. K. Lee (HKFRI 1987); 19 April 2001, S. K. Lee (HKFRI 2021); Dongdaemun-gu, Seoul, 19 April 2002, H. Y. Yun (HKFRI 1988); Dongdaemun-gu, Seoul, 16 April 2000, S. K. Lee (HKFRI 1784); Suwon, Gyeonggi, 31 March 2002, H. Y. Yun (HKFRI 1989); 13 April 2001, H. Y. Yun (HKFRI 1992); Suwon, Gyeonggi, 20 April 2001, H. Y. Yun (HKFRI 2019). III on *Juniperus chinensis* L. var. *sargentii* Henry, Jeju-si, Jeju-do, 19 April 2001, S. K. Lee (HKFRI 2019); 9 April 2002, H. Y. Yun (HKFRI 1990, 1994), 19 April 2002, S. K. Lee (HKFRI 1996); Gyeongju, Gyeongbuk, 12 April 2002, K. H. Kim (HKFRI 1991). III on *Juniperus chinensis* L. var. *globosa* Hornibr, Jeju-si, Jeju-do, 9 April 2002, S. K. Lee (HKFRI 1997). III on *Juniperus chinensis*

**Table 3.** Morphological characteristics of teliospores, aeciospores, and peridial cells of *Gymnosporangium japonicum*

Structure	Present study	Hiratsuka et al. (1992)	Kern (1973)	Lee and Kakishima (1999)
Telium	Caulicolous, on twigs, branches and trunks	Caulicolous, on twigs or branches	Caulicolous, on twigs or branches	– <sup>c</sup>
<b>Teliospore</b>				
Cell number	2	2	2	–
Shape	Ellipsoid	Ellipsoid	Ellipsoid	–
Size (µm)	13-22 × 36-70	18-24 × 46.5-70	17-25 × 57-66	–
Wall thickness (µm)	1.0-2.7	1.8-2.5	1.0-1.5	–
No. of pores	1 or 2 near the septum	2 near the septum	2 septate	–
<b>Aeciospore</b>				
Type of surface Structure	SC <sup>a</sup>	–	–	SC
Wall thickness (µm)	1.1-2.8	1.0-1.8	2.5-3.5	2.5-3.5
Size (µm)	16-21 × 20-26	15-22 × 18-28	17-19 × 19-23	17-19 × 19-23
<b>Peridial cells</b>				
Type of surface Structure <sup>b</sup>				
OW	S	–	–	S
SW	SE	–	–	SE
IW	SE	–	–	SE
Length (µm)	62-117	57-120	60-120	–

<sup>a</sup>SC: type SC (small coronate; Lee and Kakishima, 1999a).

<sup>b</sup>OW: outer wall; SW: side wall; IW: inner wall; S: type S (smooth); SE: type SE (sparsely echinulate; Lee and Kakishima, 1999b).

<sup>c</sup>Not described.

*L. var. horizontalis* Nakai, Suwon, Gyeonggi, 20 April 2001, H. Y. Yun (HKFRI 2020); Jeju-si, Jeju-do, 9 April 2001, S. K. Lee (HKFRI 1993), 19 April 2002, S. K. Lee (HKFRI 1995).

**Hosts in Korea:** O, I on *Photinia villosa* (Thunb. ex Murray) DC., *Photinia villosa* var. *brunnea* Nakai. III on *Juniperus chinensis* L., *Juniperus chinensis* L. var. *horizontalis* Nakai, *Juniperus chinensis* L. var. *sargentii* A. Henry, *Juniperus chinensis* L. var. *globosa* Hornibr.

**Geographical distribution:** This cedar-apple rust fungus was mainly collected from the northern parts of South Korea including Gyeonggi province, Kangwon province, and Gyeongbuk province.

**Remarks:** *G. japonicum* is a newly reported cedar-apple rust fungus in Korea. No significant differences in the mycological characters of *G. japonicum* were noted between the present and the previous descriptions (Table 3). Hiratsuka et al. (1992) and Kern (1973) described that telia are caulicolous and growing on twigs and branches, but telia that formed on the trunks were identified in the present study. Aecia of *G. japonicum* are known to be produced only on leaves of aecial hosts (Hiratsuka et al., 1992; Kern, 1973). However, results of this study showed that aecia are also produced on twigs of *Photinia villosa*, which could become a new character for identifying this species. As shown in Fig. 2, ultrastructural examination under the SEM revealed that surface structures of inner and side walls of

peridial cells were sparsely echinulate (type SE), which is unique to this species, compared with the other species as reported by Lee and Kakishima (1999b).

From the artificial inoculation with this fungus, a positive result was obtained only on *P. villosa* among 13 tested aecial hosts as reported in other countries (Table 1) (Kern, 1973; Hiratsuka et al., 1992), and mature aecia were obtained 70 days after inoculation. Leaves of *P. villosa* were infected by telia from *J. chinensis* in Suwon and from *J. chinensis* var. *horizontalis* in Jeju-do, and produced spermogonia and aecia. Other plant species showed no sign of spermogonia and aecia on their leaves.

In Korea, cedar-apple rust caused by *G. japonicum* has been known to rarely occur because the aecial host, *P. villosa*, naturally grows in mountainous areas in Korea, while the telial host, *J. chinensis*, is generally limited to urban areas as a landscape tree.

Specimens used in this study have been deposited at the Mycological Herbarium, The Korea Forest Research Institute, Seoul.

### Acknowledgment

The authors wish to thank Dr. Young Ho Kim of the School of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University for the valuable suggestions and editorial comments.

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